Week 1 Notebook

June 1, 2015 - June 5, 2015

June 1, 2015

Since today was our official first day in the lab, we started off the morning with a meeting and a tour of the facilities available to us in Goddard Hall. Following the meeting and the tour, we measured the DNA concentrations of minipreps that were prepared in previous weeks via UV spectrophotometry. Our spectrophotometry protocol can be found in the protocol folder. The absorbances of samples from all eight minipreps at 260 nm and 280 nm are listed in Table 1 Below. The samples are labeled according to our miniprep library.

Table 1: Abs_{260nm} and Abs_{280nm} for the minipreps made in previous weeks.

Miniprep	Abs_{260nm}	Abs_{280nm}
15-1C	0.0071	0.0058
15-2C	0.0066	0.0052
15-3C	0.0066	0.0056
15-4C	0.0054	0.0047
15-5C	0.0108	0.0091
15-6C	0.0090	0.0087
15-7C	0.0050	0.0043
15-8C	0.0060	0.0059

We then calculated the concentration of DNA in each miniprep sample using the Abs_{260nm}. An absorbance of 1.0 at 260nm indicates a DNA concentration of 50 µg/mL, so we used this value as a conversion factor. Because the sample was diluted 100 fold in the cuvette and the length of the cuvette was 0.1 cm, the equation was adjusted by a factor of 1000. Therefore, all DNA concentrations were calculated using the formula:

$$[DNA]=Abs_{260nm}*50\mu g/mL*1000$$

The calculated DNA concentrations for the 8 minipreps are listed below in Table 2.

Table 2: Concentrations of minipreps made in previous weeks.

Miniprep	DNA Concentration (µg/mL)

15-1C	355
15-2C	330
15-3C	330
15-4C	270
15-5C	540
15-6C	495
15-7C	250
15-8C	300

The minipreps were also run on gels to make sure we obtained the correct plasmids. Figures 1, 2, and 3 below show the three gels that were run. Each one only had one band, indicating that the plasmids were intact. All of the bands were of the expected size.

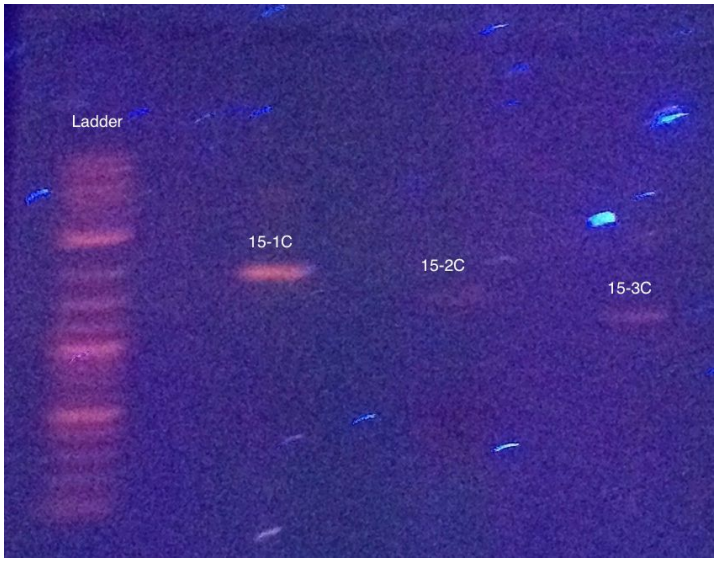


Figure 1: Gel for minipreps 15-1C through 15-3C.

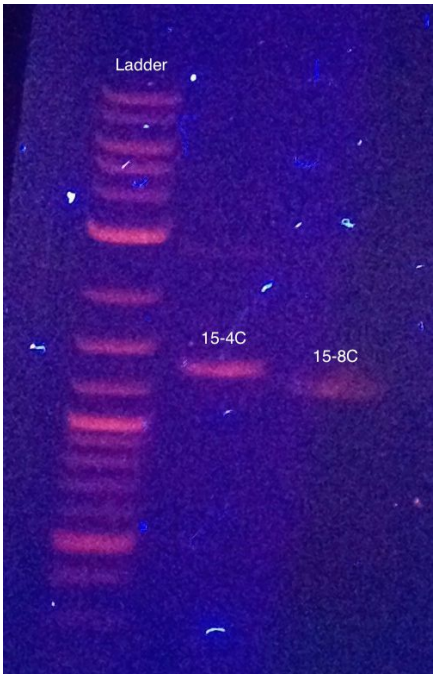


Figure 2: Gel for minipreps 15-4C and 15-8C.

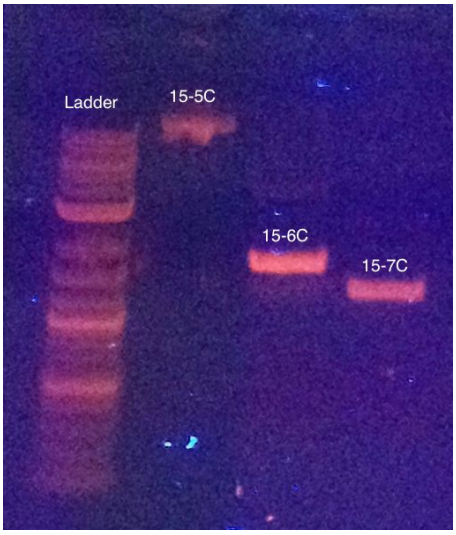


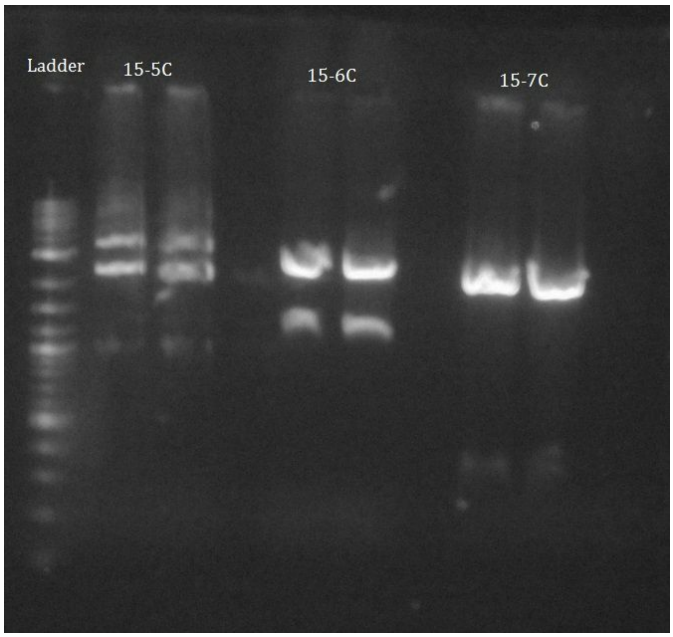
Figure 3: Gel for minipreps 15-5C through 15-7C.

June 2, 2015

Kayla/Julie

- Digest of 15-5A, 15-6C, and 15-7C with XbaI and Pst I
    - 2µg miniprep DNA, 5µL Buffer, 1 µL XbaI, 1 µL PstI, and dH_2O to 50 µL
    - Incubate at 37°C for 1 hour
- Gel purification
    - 0.9% agarose gel

- Loaded 2 wells next to each other because tape would not stick to comb to form double wide wells.
  - Lane 1=5 µL Ladder+SYBR Green
  - Lanes 2 and 3=15-5A+Loading Dye+SYBR Green
  - Lanes 4 and 5=15-6C+Loading Dye+SYBR Green
  - Lanes 6 and 7=15-7C+Loading Dye+SYBR Green
- Ran gel at 70V for 1 hour
- Cut out inserts for 15-5A and 15-6C
  - Insert band did not appear for 15-7C



- Purified according to Nucleospin Kit Protocol

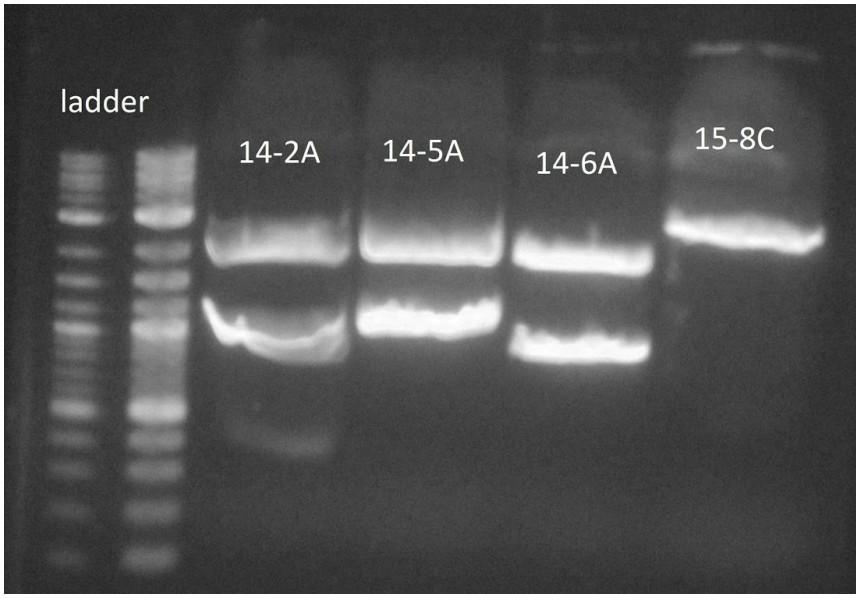
- Ligation of GFP, CFP, and RFP into J23109 vector
  - Reactions=15 µL DNA (for CFP and GFP) or 3 µL DNA (for RFP), 3 µLJ23109 vector, 1 µL ligase, 2 µL buffer, dH_2O to 20 µL
  - incubated on bench top at room temp overnight

Chloe/Charlotte

Digestion of plasmids 15-8C, 14-2A, 14-5A, 14-6A with SpeI and PstI
Following New England Biolabs protocol with the exceptions:

- 3 ul of DNA (where possible, dependent on volume available)
- Incubated for 1 hr

Gels for completed digestions: added 50 ul of purple loading dye with cyber green.



From gel: inserts from 14-2A, 14-5A, and 14-6A, and the 15-8C vector, were digested per the Nucleospin Extracts II kit protocol.

Made 500 mL 50x TAE from:

- 121 g of Tris base in 300 mL diH2O
- 28.55 g glacial acetic acid
- 50 mL 0.5 M EDTA

Ligation: J23108 plasmid with GFP, RFP (from 14-6A), and CFP inserts.

Dave/Eddie

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- Spectrophotometer used to analyze DNA Results:
    - J23109
        - 0.0175 @ 260nm
        - 0.0123 @ 280nm
        - Calculated concentration: 875 ug/ul
    - J23111
        - 0.0196 @ 260nm
        - 0.0145 @ 280nm
        - Calculated concentration: 980 ug/ul
- Conducted a digestion of J23109 & J23111 using SpeI and PstI. The following table displays the reaction conditions. Reactions were incubated at 37C for 60min.

Ingredient	J23109	J23111
Plasmid (ul)	2.29	2.04
PstI (ul)	1.0	1
SpeI (ul)	1.0	1
10x Buffer (ul)	5	5
Water (ul)	40.71	40.96
Total (ul)	50	50

- The J23109 & J23111 were then run on a gel with the following wells:
    - Well 1: Log 2 Ladder
    - Well 2: J23109
    - Well 3: J23109
    - Well 4: J23111
    - Well 5: J23111
- A gel extraction was conducted according to protocol. Gel slices were massed, and 2 gel equivalents of buffer were added. Gels were melted into the buffer at 45C for ~10 min. 30 ul of NE were used to elute the DNA.
- J23111 was ligated with each of RFP, CFP and GFP and a control was made according to the table below and stored at room temperature overnight.

Indgredient	Control	RFP	CFP	GFP
J23111 (ul)	2	2	2	2
10x Buffer (ul)	2	2	2	2
Ligase (ul)	1	1	1	1
Insert (ul)	0	3	15	15
Water (ul)	15	12	0	0
Total (ul)	20	20	20	20

- Created a poster about fluorescence for the Touch Tomorrow Event.

June 3, 2015

Kayla/Julie

- Ran transformation of overnight ligations (CFP, GFP, and RFP into J23109) following protocol provided by iGEM
    - plated 8 plates - 2 for each transformation, one with 50 µL and one with 200 µL
- Prepared CCMB80 Buffer following protocol provided by iGEM
- Worked on outreach poster

Chloe/Charlotte

Transformed competent bacteria with the plasmids from yesterday's ligation, per iGEM transformation protocol, with exceptions

- 5 ul resuspended DNA added (not 1-2)

Plated transformations--one plate 50 ul, one plate ~200 ul (the amount left over after 50 ul was removed).

Poster work for Touch Tomorrow

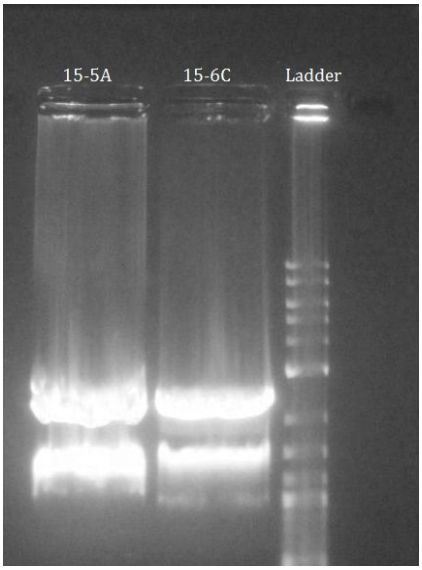
Dave/Eddie

- Prepared 4L of Cam LB plates and 0.5L of Kan LB plates according to protocol.
- Continuing from yesterday's ligation, today we transformed chemically competent bacterial cells.
    - Changes to the protocol:
    - 5ul of ligated DNA used to transform the competent cells
- Large plates were prepared in addition to standard petri dishes. The large plates are designated as canvases for TouchTomorrow or iGEM related bacterial art.
- Improved the poster for Touch Tomorrow.
- Named the computer at our lab station $_{g}$TOM (the 'g' is silent)

June 4, 2015

Kayla/Julie

- Counted colonies on transformation plates and found that none of the plates grew any colonies
    - let the plates incubate for a few more hours and checked again to find no colonies
    - let the plates incubate overnight at 37^{o}C
- Performed restriction digest on GFP (15-5A) and CFP (15-6C)
    - GFP: 11 µL DNA, 5 µL buffer, 2 µL XbaI, 2 µL PstI, and dH20 to 50 µL
    - CFP: 12 µL DNA, 5 µL buffer, 2 µL XbaI, 2 µL PstI, and dH20 to 50 µL
    - loaded 60 µL digests into gel with 10 µL ladder and ran gel at 80 V for 1.5 hours
    - Ladder did not run properly so band size could not be determined. However:
        - Each lane had a plasmid band and an insert band as expected.
        - CFP insert band was slightly larger than GFP insert band, as expected.

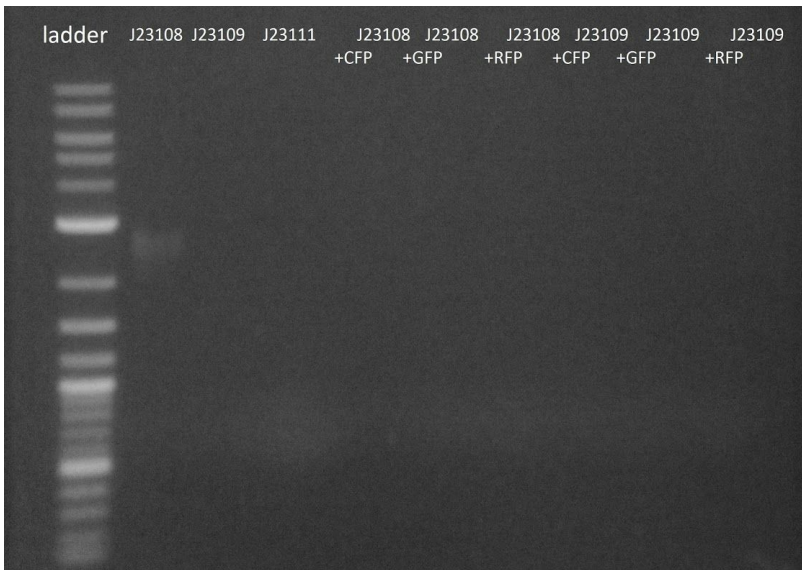


- Tested transformation with new competent cells using NEB cells and DNA
    - two DNA concentrations were used: 50 ng/μL and 5 ng/μL
    - 1 μL DNA was added to 25 μL cells
    - plated and left to incubate overnight at 37ºC
- Gel purification of GFP and CFP digests
    - According to Nucleospin Gel Purification Protocol
        - Melted gel in 42ºC water bath instead of 50ºC

Chloe/Charlotte

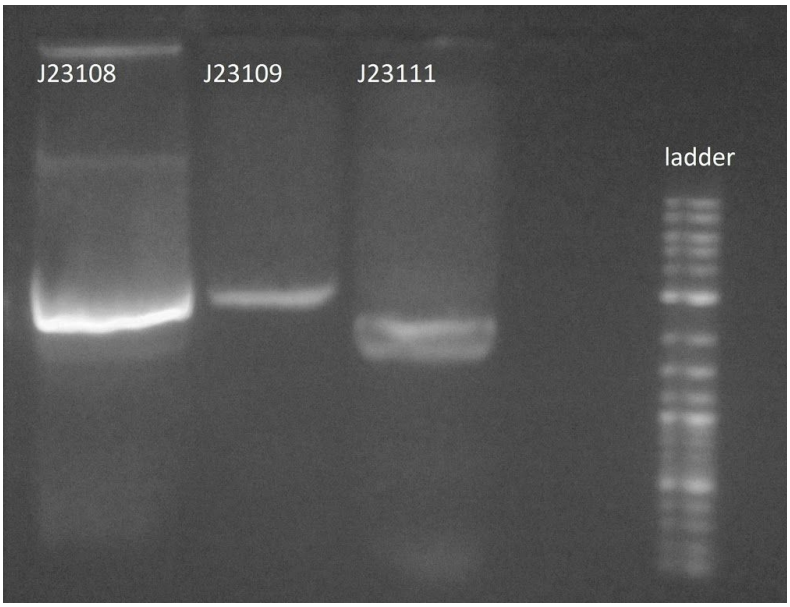
Plate check: no colony growth. Plates returned to incubator.
Ran gel for all vectors and inserts; ~3 ul used.

- Nothing observed, except for a faint band of J23108



Digestion of all 3 vectors: J23108, J23109, J23111

- 5-6 ug insert
- 2 ul SpeI
- 2 ul PstI
- 5 ul CutSmart buffer
- total volume (water added): 50 ul
- incubated at 37 C



Painting with bacteria: tried cam-resistant RFP and YFP, and kan-resistant GFP, using toothpicks, pipettes, etc.

- Picked 2 colonies each of GFP, RFP, CFP that are all 15-8C. Picked 1 colony RFP 15-10C. Colonies were suspended in LB broth and CAM and left in the shaker overnight.

Dave/Eddie

- Began experimenting with methods of 'painting' with fluorescent E. coli. Cultures did not fluoresce under UV light as expected, cause unknown. Regardless, we attempted to use paintbrushes, a toothpick, a syringe, a pipette tip, and a hockey stick to illustrate on standard plates. Smaller paintbrushes worked significantly better than the indelicate large brushes, and syringes proved too quick to dispense liquid to be useful. However, the hockey stick, toothpick, and pipette tip all seemed to work very well. We also conducted a dilution experiment to determine the ideal concentration for painting. The CAM plate was divided into grid zones, grids as follows. Each grid contains 5ul spread with the tip of a hockey stick. RFP+YFP was created by mixing equal volumes of the

appropriate volumes of both cultures. The GFP was cloned into a different vector, and had to be plated on a KAN plate instead.

	CAM plate		KAN plate
A	RFP no dilution	A	GFP no dilution
B	RFP 1:10	B	GFP 1:10
C	RFP 1:100	C	GFP 1:100
D	RFP 1:20	D	GFP 1:20
E	RFP 1:5	E	GFP 1:5
F	YFP no dilution		
G	YFP 1:10		
H	YFP 1:100		
I	YFP 1:20		
J	YFP 1:5		
K	RFP+YFP no dilution		
L	RFP+YFP 1:10		
M	RFP+YFP 1:100		
N	RFP+YFP 1:20		
O	RFP+YFP 1:5		

- Colonies that grew from yesterday's plates:

Plate	# of colonies
15-9C RFP 50ul	25
15-9C RFP 200ul	140
15-9C CFP 50ul	0
15-9C CFP 200ul	0
15-9C GFP 50ul	0

15-9C GFP 200ul	0
15-9C No insert 50ul	4
15-9C No insert 200ul	7

- We are picking colonies from the RFP and leaving the other plates to incubate overnight again. 6 colonies were picked from the 200ul RFP plate.
- 15-4C was digested using XbaI and PstI to be combined with different promoters. 15-4C is an AFP from the 2015 stocks. We set up a gel purification but used the loading dye without SYBR green, so weren't able to extract the DNA. We will repeat this tomorrow the right way.
- 14-5A was transformed for the same purpose, 14-5A is the strong constitutive promoter J23100.
    - Transformation of 14-5A

June 5, 2015

Kayla/Julie

- Ligation of GFP and CFP into J23108, J23109, and J23111 vectors
    - Reactions=3 µLgel purified DNA, 3 µL vector, 1 µL ligase, 2 µL buffer, dH_2O to 20 µL
    - incubated on bench top for 2 hours at room temperature
- Checked test transformation plates and GFP/CFP/RFP transformation plates from 6/3/15
    - No colonies on test transformation plates
    - 1 colony on 1 RFP+15-10C plate but no colonies on rest
- Checked RFP/YFP plates used to make art!
- Transformed with 5 uL of each ligation
    - Following iGEM protocol
    - Plated on 12 Cam plates (50 uL on 1 and 250 uL on another for each of the 6 transformations)
- Prepared M9 minimal media for biofilm assay
    - Prepared M9 salts using 64g Na_2HPO_4-$7H_2O$, 15g KH_2PO_4, 2.5g NaCl, 5.0g NH_4Cl, Adjust to 1000ml with distilled H_2O and sterilize by autoclaving
    - Prepared other components
        - 20% D-glucose using 20g D-(+)-glucose in 100 mL water
        - 10g thiamine in 100 mL water
        - 50 mL of 1 M CaCl2
        - 50 mL of 1 M MgSO4
    - Measure ~700ml of distilled H_2O (sterile)
    - Add 200ml of M9 salts
    - Add 2ml of 1M $MgSO_4$ (sterile)

    - Add 20 ml of 20% glucose (or other carbon source)
    - Add 100ul of 1M $CaCl_2$ (sterile)
    - Adjust to 1000ml with distilled H_2O
- Prepared minimal media for biofilm assay
- Prepared LB media

Chloe/Charlotte

The plasmids that were grown in *E. coli* cultures overnight were added to the library of parts as 15-11A through 15-14A.

Miniprep: used NucleoSpin kit and followed manufacturer's protocol to miniprep cultures of *E. coli* containing: 15-11A (2 colonies), 15-12A (2 colonies), 15-13A (2 colonies), 15-14A (1 colony).

Test digest carried out, using:

- 1 ug miniprep
- 2 ul 10x buffer (CutSmart)
- 1 ul EcoRI
- 1 ul PstI
- water added to give total volume of 20 ul
- incubated in 37 C water bath for 1 hour

Dave/Eddie

- Upon reviewing the paintings, it seems that the hockey stick and pipette tip are both bad options, leaving holes in the designs. The toothpick or small brush seem to be the best options.
- 15-4C was digested again using XbaI and PstI and gel purified.
- Conducted a miniprep of the six RFP colonies that were left to incubate overnight. Protocol was followed without the optional step.
- Spectrophotometer was used to analyze DNA concentration:

Sample	Absorbance @260	Absorbance @280	Concentration (ug/ul)
1	0.0037	0.0027	185
2	0.0214	0.0148	1070
3	0.0176	0.0128	880
4	0.0031	0.0005	155
5	0.0032	0.0012	160
6	0.0085	0.0058	425

- Performed a test digestion of 15-9C and ran a gel for the six colonies.

June 6, 2015

Kayla/Julie

- Checked GFP and CFP transformation plates
- Colony numbers

Vector and Insert	50 µL Plate	200 µL Plate
J23108 without insert	>100	>100
J23108+GFP	>100	>100
J23108+CFP	>100	>100
J23109 without insert	0	1
J23109+GFP	8	13
J23109+CFP	1	5